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A Service for Human Chromosome Studies in Saskatchewan

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ABSTRACT

A service has been developed in Saskatchewan to make available the results of studies of human chromosomes, the material being forwarded to the laboratory by local transport facilities. During the first year of this project chromosome studies were requested for five doubtful cases of trisomy-21 (two were found to be normal) and for 20 definite cases of trisomy-21 in young patients (two had translocations but the parents of both these children had normal karyotypes). Eleven confirmed cases of Turner's syndrome, two of Klinefelter's syndrome, and one each of the D and E syndromes were also studied. The largest group for which studies were requested comprised 36 patients with mental retardation; only two abnormal karyotypes were encountered in this group.

SOMMAIRE

En Saskatchewan, on a mis sur pied un service destiné à l'étude des chromosomes humains, les spécimens étant envoyés au laboratoire par les moyens de transport locaux. An cours de la première année d'application de ce projet, le service a été prié d'étudier cinq cas douteux de trisomie-21 (dont deux se sont révélés normaux) et 20 cas nets de trisomie-21 chez des jeunes malades (on a trouvé des translocations chez deux des enfants, mais leurs parents avaient tous deux des cariotypes normaux). On a également étudié 11 cas confirmés de syndrome de Turner, deux de Klinefelter et un cas de chacun des syndromes D et E. Le groupe le plus important pour lequel des études ont été demandées comprenait 36 arriérés mentaux; dans ce groupe, on n'a trouvé que deux cariotypes anormaux.

THE study of human chromosomes is an essential component in the investigation of a number of disorders and plays a confirmatory role in the study of others. The culture of lymphocytes, however, and the preparation and analysis of karyotypes require well-trained personnel and meticulous attention to detail. It is not the type of work which, at the moment, lends itself to routine mass production methods. In order to make such studies available in Saskatchewan a laboratory was set up in the Department of Pediatrics of the University of Saskatchewan, the equipment being provided by the Saskatchewan Association for Retarded Children and running costs being covered by a Provincial Health Grant. Having established the laboratory, it then became necessary to devise methods that would make possible studies on patients outside the city of Saskatoon, because many patients would remain unstudied if they had in the first instance to be transported to Saskatoon. These studies also had to be carried out on small quantities of blood, for among the cases requiring study would be newborn babies with multiple anomalies and suspected mongolism; large quantities of blood would be difficult if not technically impossible to obtain from such infants in outlying hospitals.

To meet these requirements a micromethod, using whole blood from a heel or finger prick only, based on the methods of Arakaki and Sparkes[1] and Ho and Smith,[2] was developed. We believe that the method used may be of interest to others anxious to develop similar services to cover a wide geographic area. The procedure is as follows.

MATERIALS AND METHODS

When a doctor encounters a problem which he thinks merits chromosomal study, he discusses the case by telephone with the cytologist in charge of the laboratory or with the geneticist. If it appears that chromosomal studies are indicated, the following procedure is carried out:

1. Four Rockefeller tubes, each containing the following culture media, are prepared: TC199 (Difco), 4 ml.; fetal calf serum (Hyland Laboratories), 1 ml.; penicillin, 0.1 ml. (400 I.U.); and streptomycin, 0.1 ml. (500 μg.). The pH is adjusted to 7.2-7.6 with CO_2 gas (5% in air) or $NaHCO_3$ (7.5%).

2. These materials, together with lancets and heparinized micro-blood-collecting tubes, are then despatched by bus to the doctor concerned. The media are kept refrigerated overnight. The following day, blood is obtained from the patient. The skin is first cleansed with alcohol and allowed to dry; it is then stabbed with the lancet so that blood flows freely. The blood is allowed to run up the micro-blood-collecting tube until it is nearly full (approximately 0.2-0.3 ml.) and after 30 seconds it is blown gently into one of the Rockefeller tubes

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containing the culture media. A second micro-blood-collecting tube is similarly filled and emptied into a second Rockefeller tube. When blood has been added to each Rockefeller tube in this way, the tubes are tightly closed with non-toxic rubber stoppers and are gently agitated to mix the blood with the medium. The tubes are then carefully packed and returned to the laboratory by bus, care being taken in winter to ensure that they are transported inside the vehicle.

For adults and older children it is often less painful and simpler to withdraw 2 ml. of blood from a vein into a syringe previously rinsed with heparin solution, 1000 units/ml., and then to introduce 0.3 ml. of the blood so obtained into each of the Rockefeller tubes.

3. On arrival at the laboratory, 0.1 ml. of solution of phytohemagglutinin (Burroughs Wellcome) is added to each tube, and the latter are then incubated for three days at 37° C. During incubation the pH is adjusted if necessary.

4. After three days (65 hours), desacetyl-methyl-colchicine (Colcemid, Ciba), 0.1 ml. of 0.04% solution, is added to each Rockefeller tube and gently mixed by swirling, and the tubes are allowed to incubate for a further four to five hours. This strength of desacetyl-methyl-colchicine is obtained by diluting a 1-ml. ampoule (strength 1 mg. in 1 ml.) with 1.5 ml. sterile distilled water.

5. At the end of incubation the cells are pipetted into suspension and then transferred to a centrifuge tube (13 ml.).

6. The suspension is spun down at 800 r.p.m. for 10 minutes.

7. The supernatant is removed and prewarmed (37° C.) hypotonic solution (one part Hank's solution and four parts distilled water) is added and the suspension is incubated for 15 minutes.

8. It is then spun at 600 r.p.m. for 10 minutes and the supernatant is again removed.

9. Two ml. fixative (three parts methanol and one part glacial acetic acid), prepared just before use, is introduced along the inside wall of the tube, drop by drop, without disturbing the cells, and the preparation is fixed for at least 30 minutes at room temperature.

10. The fixative is replaced with freshly made fixative and the cells are broken up into suspension by pipetting. The suspension is allowed to stand for 10 to 20 minutes.

11. It is then spun again at 600 r.p.m. for 10 minutes and the fixative is changed (the amount added depending on the density of cell suspension required).

12. An acid-clean slide (previously soaked in cleaning solution made up of sodium dichromate and sulfuric acid, for one hour) is rinsed under cold tap water, the excess water is shaken from the slide, and two to three drops of cell suspension are pipetted on to the water-filmed slide. The slide is tilted immediately and the excess water is blotted from it. Dryness can be hastened by warming briefly over a spirit flame.

13. The slide is then examined with the phase contrast microscope for chromosome spreading. If the chromosomes are not well spread the fixative is changed one or two more times, using freshly made fixative.

14. Slides are stained in 1% aceto-orcein (50% glacial acetic acid) for 30 seconds, passed through two changes of absolute alcohol and two changes of xylol and then mounted in permount.

15. Cells with good spreading of chromosomes are counted and photographed under phase contrast. Microfile film with high contrast is used and developed in 1:1 Dekotol for five minutes, fixed for 20 minutes in acid fixer and washed for one hour in cold tap water before drying. Pictures are printed with a Kenora copying machine, using No. 3 paper.

16. Chromosome figures are individually cut out and arranged according to both Denver and Patau systems, and pasted on bristol board. Photostat copies are made and sent together with the formal report to the doctors concerned.

RESULTS

During the first year of operation of this service the laboratory studied 71 cases. These have fallen into five main groups:

1. *Trisomy-21 and suspected trisomy-21* (mongolism, Down's syndrome, G-trisomy).

Diagnosis.—There is sometimes considerable doubt in the mind of the family physician, obstetrician and/or pediatrician regarding the diagnosis of trisomy-21 in the newborn period; in one instance in our experience the problem was heightened by the fact that the baby was of Chinese extraction. Chromosomal studies are very helpful in excluding or confirming the diagnosis and were carried out on this account in five instances. The diagnosis may also sometimes be questioned in older children; in one such case, preparations were studied from both blood and skin and the child was found not to have trisomy-21. Blood was studied from two others who had features of trisomy-21 but who were unexpectedly intelligent. Contrary to expectation, the brighter child with an intelligence quotient of 90 had the typical trisomy-21, while the second child, who had an intelligence quotient of 75, showed a mosaic pattern.

Counselling.—More commonly the diagnosis is not in doubt, but if the mother is young, studies are requested to determine whether or not the infant is a so-called "translocation mongol". If a translocation is found, the parents are studied to determine whether or not one or the other is a translocation carrier and is therefore more likely than a normal parent to produce another baby with such

a syndrome. Out of a total of 20 cases of trisomy-21 studied, two cases involving translocation, both of the G/G type, were detected, but in each instance both parents had normal karyotypes; the translocation was therefore sporadic and had arisen for the first time in the affected babies. One family with only two children, both of whom had trisomy-21 of the usual type, was also studied; in this family both parents had normal karyotypes but were over 40 years old when their children were born.

2. *Girls with Unexplained Dwarfism and/or Infantilism*

All girls with unexplained dwarfism, whether they have or do not have the stigmata of Turner's syndrome (puffy hands and feet, webbing of the neck, wide carrying angles at the elbows etc.), and those with unexplained infantilism (no breast development and primary amenorrhea) should at least be subjected to a buccal smear. If the latter is chromatin-negative, the diagnosis of Turner's syndrome will be confirmed. If it is positive, the patient may still present a variant of Turner's syndrome, having either some variety of mosaicism (e.g. XO/XX, XO/XX/XXX) or one normal and one abnormal X chromosome (e.g. partial deletion or isochromosome). Eleven such patients were studied; 10 of these had an XO and one had an XO/XX karyotype.

3. *D and E Syndromes*

The clinical features of babies with these syndromes were recently outlined by Chute[3] and though the diagnosis can usually be made on clinical grounds ,it needs chromosomal confirmation, not only to establish the diagnosis but also to determine whether the baby in question has a straightforward trisomy in either the D or E group of chromosomes or has a translocation. If the baby has a translocation, it will be necessary for the parents to be studied, for one or other may be a balanced translocation carrier. Should this prove to be the case, there will be an increased risk that any baby born subsequently to these parents will also be affected, and the parents should be counselled accordingly.

The recurrence risk among the siblings will theoretically depend mainly on whether the chromosomes involved in the translocation are homologous or non-homologous. In the case of D/D translocation, if homologous chromosomes (e.g. D_1/D_1) are involved, then all the offspring should be affected. Otherwise (e.g. D_1/D_2) only one-third should be affected. The latter condition would be similar to the D/G translocation in trisomy-21 where it has been observed through progeny studies on reported families that the mother carrier will give birth to approximately equal proportions of normal, balanced carrier and affected offspring as theoretically expected.[4] However, if the carrier is the father the situation is different. Here the ratio is 20:20:1 respectively. The reason for the disproportion is not clearly understood, and so far it is not known whether in this condition a similar situation prevails, as insufficient families with D group translocations have been reported. Unless unaffected children have already been born in the family, it is not possible to tell whether or not involved chromosomes are homologues. To overcome this difficulty cytological techniques have been developed, using autoradiography, that may make it possible to identify the chromosomes involved through their differential DNA synthesis patterns.

Two babies in this group have been studied; one was a D/D interchange trisomic, the other a regular E trisomic, involving chromosome 18.

4. *Klinefelter's Syndrome*

It has been observed that about one per 500 live-born males are chromatin-positive while less than one in 2000 live-born females are chromatin-negative[5] and one in 600 live-born babies have trisomy-21.[4] Among the infants born in Saskatchewan each year, therefore, we should expect about 10 cases of chromatin-negative Turner's syndrome, about 40 cases of trisomy-21, and 40 cases of Klinefelter's syndrome. However, only two cases of the latter syndrome were referred to us for study. Some of these cases will come to light only if routine buccal smears are carried out on all mentally retarded males. However, since not all patients with Klinefelter's syndrome are mentally retarded, many more subjects should be detected if, at a routine premarital examination, all males were adequately examined, including palpation of the testes and, if the latter are small, a buccal smear.

5. *Patients with Mental Retardation and Multiple Congenital Anomalies*

By and large most patients with multiple congenital anomalies, apart from those already known to be associated with a chromosomal anomaly (e.g. trisomy-21, D or E syndrome), have normal karyotypes. Nevertheless any such patient who does not show the characteristics of a well-recognized syndrome should, in the present state of our knowledge, have chromosome studies carried out when possible, for it is only by this means that new chromosome syndromes will be discovered. The yield from such a harvest is small. We have studied 36 such patients; 34 had normal karyotypes and two had not. These two probably provide the most interesting material that has passed through our hands, and will be the subject of a separate communication.

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